" Synthetic peptides useful in biological essays for detecting infections caused by group O HIV-1 viruses"

The invention relates to synthetic peptides which can be used in biological tests for the detection of infections due to the group O HIV-1 viruses, to the method for preparing them, to compositions and kits containing such peptides and to the biological tests using such peptides.

Group O HIV-1 retroviruses are known in the prior art. Patent EP 0,345,375 and patent application EP 0,657,532 describe the ANT 70 and ANT 70 NA isolates isolated from Cameroonian patients. These documents describe more precisely antigens and antigenic compositions containing lysates or proteins of these isolates, the nucleic acids corresponding to the genomic RNA, hybridization methods using these nucleic acids, methods of producing the isolates indicated above as well as methods of preparing p12, p16, p25, gp41 and gp120 proteins of these retroviruses.

Application EP 0,591,914 describes the MVP 5180/91 isolate. This isolate, characterized by Western blotting, exhibits, like the previous isolate, differences in relation to the HIV-1 retrovirus isolates which have been known for a long time. Application EP 0,591,914 describes precisely the DNA sequence of the MVP 5180/91 isolate and indicates precisely the location of the gag, pol and env genes. Application EP 0,591,914 further describes synthetic peptides of the V3 loop as well as the immunodominant region (gp41). They are useful for biological tests, in particular for the *in vitro* detection of group O HIV-1 antibodies.

Sub. E1

Application EP 0,673,948 describes synthetic or recombinant peptides consisting of 15 to 50 amino acids (AA) and comprising the sequence

SEQ ID no

— -VWGIRQLRARLQALETLIQNQQRLNLWG**X**KGKLI**X**YTSVKWNTSWSGR-

in which X represents either a cysteine residue, or a serine residue. These peptides are useful in the diagnostic field for the detection of infections due to certain group O HIV-1 retrovirus isolates.

Application EP 0,727,483 is also known which describes the MVP 2901/94 isolate which also forms part of the retroviruses belonging to the group O HIV-1 family. This application describes certain antigens having well-determined peptide sequences. These peptide sequences correspond to part of the sequence of gp120 and part of gp41 (immunodominant region) of the MVP 2901/94 isolate.

Application WO 96/12809 describes two new isolates belonging to the group O HIV-1 family. They are the VAU and DUR isolates. This application describes certain peptide sequences derived from the two viruses cited above, which are useful for the detection of antibodies recognizing the HIV-1 VAU or DUR peptide sequences.

Application WO 96/32293 describes two antigens derived from the sequence of the ANT 70 isolate. They are the antigen called MDL061 and the antigen MDL056, of the immunodominant region of gp41. According to this invention, to detect 100% of the samples of a limited collection of sera from patients infected with the group O HIV-1 virus, it is necessary to use compositions containing these two peptides, since each isolated peptide does not allow, on its own, satisfactory results to be obtained.

Indeed, it is practically impossible, in the light of the genetic variability shown by the isolates of the group O virus, to guarantee serological screening of individuals infected by the use of antigens derived from the same and sole isolate. This means that it is not possible to obtain reagents which guarantee 100% sensitivity. The O group thus raises, for the first time, a major problem; it is the inability of certain serological reagents to recognize individuals infected with particularly divergent groups or subtypes. This is precisely the case for the group O HIV-1 viruses.

Application WO 96/40763 also stresses the great divergence of the O group. This application describes peptides which incorporate, into a natural HIV-1 type B sequence, a few minor modifications (replacement of one or two amino acids). According to this application, these hybrid peptides are capable of reacting with anti-group O antibodies.

Application WO 96/27013 describes a series of new group O HIV-1 viruses designated BCF 01, BCF 02, BCF 03, BCF06, BCF 07, BCF 08, BCF09, BCF11, BCF12, BCF13 and BCF14 as well as a series of peptides of the corresponding gp41 dominant region which are called ESS/BCF02, FAN/BCF01, LOB/BCF06, MAN/BCF07, NKO/BCF08, POC/BCF03, NAN/BCF11, BCF09, BCF12, BCF13 and BCF14. A number of these peptides are difficult to handle in diagnosis because of their low solubility, especially the peptide BCF13.

Unexpectedly, it has now been found that certain synthetic peptides are diagnostic reagents of superior quality and they make it possible to satisfactorily screen patients infected with group O HIV-1 retroviruses. These peptides are composed of variable sequences articulated around highly conserved short sequences, which are present in isolates of the group O HIV-1 retroviruses. The peptides of the invention make it possible to obtain results which are quite superior to those obtained with synthetic peptides carrying immunodominant epitopes of the gp41 (env) of certain group O HIV-1 isolates.

Subsequently, to name the amino acids, the three-letter nomenclature will be used.

The synthetic peptides of the invention correspond to the general formula (I): (Various regions of SEQ ID NOS 1-16, respectively)

Δ-Z-TrpGlyCys-Θ-CysTyrThrSer-Ω (I)

in which :

-Δ represents a biotinyl radical, a biocytinyl radical, a hydrogen atom, an acetyl (CH_3CO-) radical, an aliphatic chain which may contain one or two thiol, aldehyde or amine functional groups, the aliphatic chain preferably being an alkyl chain of 1 to 6 carbon atoms or an alkenyl chain of 2 to 6 carbon atoms, or an aminoalkylcarbonyl chain of 2 to 6 carbon atoms,

-Z represents a peptide sequence of one of the formulae (II) to (X) :

$-\Xi_1$-Ser-Ξ_2-	(II)
-Ser-Ξ_2-	(III)
$-\Xi_1$-Ser-	(IV)
$-\Xi_1$-Gln-Ξ_2-	(V)
-Gln-Ξ_2-	(VI)
$-\Xi_1$-Gln-	(VII)
$-\Xi_1$-Asn-Ξ_2-	(VIII)
-Asn-Ξ_2-	(IX)
Ξ_1-Asn-	(X)

in which :

-Ξ_1 represents a peptide sequence of 0 to 9 amino acids and

-Ξ_2 represents a peptide sequence of 0 to 5 amino acids,

-Θ represents a peptide sequence of formula (XI):

$$-(AA_1)-(AA_2)-(AA_3)-(AA_4)-(AA_5)- \quad (XI)$$

in which :

- (AA_1) represents either a lysine residue, or an arginine residue, or an ornithine residue,
- (AA_2) represents either a glycine residue, or an asparagine residue,
- (AA_3) represents either a lysine residue, or an arginine residue, or an ornithine residue,
- (AA_4) represents either a leucine residue, or an alanine residue, or an isoleucine residue, or a glutamine residue,

• (AA_6) represents either an isoleucine residue, or a valine residue, or a leucine residue, or a threonine residue, or a norleucine residue, or a norvaline residue,

provided, however, that (AA_1), (AA_2), (AA_3), (AA_4) and (AA_5) never form together the peptide sequences -Lys Gly Lys Leu Ile- and -Lys Gly Lys Leu Val-,

-Ω, attached to the -CO- group of serine, represents:

- a hydroxyl (-OH) radical or an amino ($-NH_2$) radical,
- an alkoxy radical comprising from 1 to 6 carbon atoms,
- a peptide sequence of formula (XII) :

-Val-Σ-Ψ (XII)

in which Σ represents a sequence of formula (XIII) or of formula (XIV) :

-(AA_6)-Trp Asn-(AA_7)-(AA_8) (XIII)

-(AA_6)-Trp His-(AA_7)-(AA_8) (XIV)

in which :

- (AA_6) represents an amino acid different from lysine,
- (AA_7) represents an amino acid,
- (AA_8) represents a serine or threonine residue,

and Ψ, attached to the -CO- residue of the free AA_8 amino acid, represents an OH or NH_2 group or an alkoxy radical comprising from 1 to 6 carbon atoms,

- a peptide sequence of formula (XV) :

-Val-Ψ (XV)

in which Ψ, attached to the -CO- residue of valine, has the same meaning as for the formula (XII),

- or a peptide sequence of one of the formulae (XVI) to (XVIII) :

-Z-TrpGlyCys-Θ-CysTyrThrSer-Ψ (XVI)

Val-Σ-Z-TrpGlyCys-Θ-CysTyrThrSerVal-Σ-Ψ (XVII)

Val-Z-TrpGlyCys-Θ-CysTyrThrSerVal-Ψ (XVIII)

in which Z and Θ have the definition given for the formula (I)

and Σ has the definition given for the formula (XII) and Ψ, attached to the -CO- residue of serine, on the -CO- residue of the AA_8 amino acid or on the -CO- residue of valine, has the same meaning as for the formula (XII).

When Ω represents a peptide sequence of one of the formulae (XVI) to (XVIII), the peptide of formula (I) becomes a dimer whose size may vary from 26 to 66 amino acids. When Ω does not represent a peptide sequence of one of the formulae (XVI) to (XVIII), the peptides of formula (I) are of the monomeric type and their size may vary from 13 to 33 amino acids.

The peptides according to the invention may be either in a linear form, or in a form cyclized by means of inter-cysteine disulphide bridges.

The compounds of formula (I) in which (AA_5) represents either a valine residue, or a leucine residue, or a threonine residue are preferred, and when Ω corresponds to a peptide sequence of formula (XII), (AA_6) represents either a glutamine residue or an arginine residue.

The peptides of formula (I) are preferred in which:

-Δ represents a biotinyl radical, a hydrogen atom or an aliphatic chain which may contain one or two thiol, aldehyde or amine functional groups, the aliphatic chain preferably being an alkyl chain of 1 to 6 carbon atoms, or an aminoalkylcarbonyl chain of 2 to 6 carbon atoms,

-Z represents a peptide sequence of formula (II) or (V), in which Ξ_1 represents a peptide sequence of two amino acids and Ξ_2 represents an amino acid, or a sequence of formula (IV), in which Ξ_1 represents three amino acids, or a peptide sequence of formula (VIII), in which Ξ_1 represents a peptide sequence of nine, eight or three amino acids and Ξ_2 a peptide sequence of fiv amino acids,

-Θ represents a peptide sequence of formula:

-Lys Gly Arg Leu Val-,

-Arg Gly Lys Ala Val-,

- Arg Gly Arg Leu Val-,

or

-Arg Gly Arg Ala Val-

and

-Ω represents a hydroxyl group, the peptide sequence (XV) or one of the following sequences which correspond to the peptide sequence of formula (XII) :

- Val Arg Trp Asn Glu Thr-Ψ,

- Val Gln Trp Asn Glu Thr-Ψ

or

- Val Gln Trp Asn Ser Thr-Ψ

Preferably, Z represents a peptide sequence of formula:

- -Leu Leu Ser Ser-
- -Leu Leu Asn Ser-
- -Leu Leu Gln Ser-
- -Arg Leu Asn Ser-
- -Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asn Ser-
- -Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asp Leu-
- -Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asn Ile-
- -Leu Asn Gln Gln Arg Leu Leu Asn Ser-

or

- -Arg Ala Leu Glu Thr Leu Leu Asn Gln Gln Arg Leu Leu Asn Ser-

Also forming part of the invention are the synthetic peptides comprising from 20 to 50 amino acids and corresponding to the formula (Ia) :

$$\Delta\text{-}Z_a\text{-TrpGlyCys-}\Theta\text{-CysTyrThrSer-}\Omega_a \qquad \text{(Ia)}$$

in which Z_a represents a radical of formulae IIa to Xa :

Ξ_{1a}-Ser-Ξ_{2a} (IIa)

-Ser-Ξ_{2a} (IIIa)

-Ξ_{1a}-Ser (IVa)

Ξ_{1a}-Gln-Ξ_{2a} (Va)

-Gln-Ξ_{2a} (VIa)

Ξ_{1a}-Gln- (VIIa)

Ξ_{1a}-Asn-Ξ_{2a} (VIIIa)

-Asn-Ξ_{2a} (IXa)

-Ξ_{1a}-Asn (Xa)

in which :

-Ξ_{1a} represents a peptide sequence of 1 to 5 amino acids and

-Ξ_{2a} an amino acid,

-Ω_a represents a peptide sequence of formula (XII), as defined for the formula (I), or a peptide sequence of formula (XVIIa) : (various regions of SEQ ID NOS 1-16, respectively)

Val-Σ-Z_a-TrpGlyCys-Θ-CysTyrThrSerVal-Σ-Ψ (XVIIa)

in which Z_a has the meaning given for the formula (Ia) and

Δ, Θ, Σ and Ψ have the same meaning as for the formula (I).

The peptides of formula (I) or (Ia) including one of the following sequences (these peptides may be of the dimer type or of the monomer type as defined above) are preferred. The sequences are given according to the one- and three-letter nomenclatures:

Sequence No. 1

-LLSLWGCRGKAVCYTSVQWNET-

or

-Leu Leu Ser Leu Trp Gly Cys Arg Gly Lys Ala Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr-
22

Sub. E3

Sequence No. 2

-LLSLWGCRGRLVCYTSVQWNET-

or

-Leu Leu Ser Leu Trp Gly Cys Arg Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr-
22

Sequence No. 3

-LLSSWGCKGRLVCYTSVQWNET-

or

-Leu Leu Ser Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr-
22

Sequence No. 4

-LLSSWGCKGRLVCYTSVQWNST-

or

-Leu Leu Ser Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Ser Thr-
22

Sequence No. 5

-LLQSWGCKGRLVCYTSVQWNST-

or

-Leu Leu Gln Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20
Ser Thr-
22

Sub. E3

Sequence No. 6

-LLNSWGCRGKAVCYTSVQWNET-

or

-Leu Leu Asn Ser Trp Gly Cys Arg Gly Lys Ala Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20
Glu Thr-
22

Sequence No. 7

-LLSLWGCRGRAVCYTSVQWNET-

or

-Leu Leu Ser Leu Trp Gly Cys Arg Gly Arg Ala Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20
Glu Thr-
22

Sequence No. 8

-LLSSWGCRGRLVCYTSVQWNET-

or

-Leu Leu Ser Ser Trp Gly Cys Arg Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20
Glu Thr-
22

Sequence No. 9 :

-LLSSWGCKGRLVCYTS-

or

-Leu Leu Ser Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser-

1 5 10 15

Sub. B3

Sequence No. 10 :

-LLNSWGCKGRLVCYTS-

or

-Leu Leu Asn Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser-

1 5 10 15

Sequence No. 11 :

-ALETLLQNQQLLNSWGCRGRLVCYTSVRWNET-

or

-Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asn Ser Trp Gly Cys Arg Gly

1 5 10 15

Arg Leu Val Cys Tyr Thr Ser Val Arg Trp Asn Glu Thr-

20 25 30

Sequence No. 12 :

-ALETLLQNQQLLNIWGCRGRLVCYTSVRWNET-

or

-Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asn Ile Trp Gly Cys Arg Gly

1 5 10 15

Arg Leu Val Cys Tyr Thr Ser Val Arg Trp Asn Glu Thr-

20 25 30

Sequence No. 13:

-ALETLLQNQQLLDLWGCRGRLVCYTSVRWNET-

or

-Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asp Leu Trp Gly Cys Arg Gly
1 5 10 15

Arg Leu Val Cys Tyr Thr Ser Val Arg Trp Asn Glu Thr-
20 25 30

Sub. E3

Sequence No. 14:

-LNQQRLLNSWGCKGRLVCYTSV-

or

-Leu Asn Gln Gln Arg Leu Leu Asn Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr
1 5 10 15

Thr Ser Val-
20

Sequence No. 15:

-RALETLLNQQRLLNSWGCKGRLVCYTSV-

or

- Arg Ala Leu Glu Thr Leu Leu Asn Gln Gln Arg Leu Leu Asn Ser Trp Gly Cys Lys
1 5 10 15

Gly Arg Leu Val Cys Tyr Thr Ser Val-
20 25

Sequence No. 16:

-RLNSWGCKGRLVCYTSV-

or

- Arg Leu Asn Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val-
1 5 10 15

The synthetic peptides below are particularly preferred peptides:

PEPTIDE No. 1 (2B) : SEQ ID No. 1

LLSLWGCRGKAVCYTSVQWNET

or

Leu Leu Ser Leu Trp Gly Cys Arg Gly Lys Ala Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr
22

Sub. E3

PEPTIDE No. 2 (3B) : SEQ ID No. 2

LLSLWGCRGRLVCYTSVQWNET

or

Leu Leu Ser Leu Trp Gly Cys Arg Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr
22

PEPTIDE No. 3 (4B) : SEQ ID No. 3

LLSSWGCKGRLVCYTSVQWNET

or

Leu Leu Ser Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr
22

PEPTIDE No. 4 (5B) : SEQ ID No. 4

LLSSWGCKGRLVCYTSVQWNST

or

Leu Leu Ser Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Ser Thr
22

PEPTIDE No. 5 (6B) : SEQ ID No. 5

LLQSWGCKGRLVCYTSVQWNST

or

Leu Leu Gln Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Ser Thr
22

Sub. E3

PEPTIDE No. 6 : SEQ ID No. 6

LLNSWGCRGKAVCYTSVQWNET

or

Leu Leu Asn Ser Trp Gly Cys Arg Gly Lys Ala Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr
22

PEPTIDE No. 7 : SEQ ID No. 7

LLSLWGCRGRAVCYTSVQWNET

or

Leu Leu Ser Leu Trp Gly Cys Arg Gly Arg Ala Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr
22

PEPTIDE No. 8 (7B) : SEQ ID No. 8

LLSSWGCRGRLVCYTSVQWNET

or

Leu Leu Ser Ser Trp Gly Cys Arg Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr
22

PEPTIDE No. 9 (12B) : SEQ ID No. 9

LLSSWGCKGRLVCYTS

or

Leu Leu Ser Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser
1 5 10 15

Sub. E3

PEPTIDE No. 10 (14B) : SEQ ID No. 10

LLNSWGCKGRLVCYTS

or

Leu Leu Asn Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser
1 5 10 15

PEPTIDE No. 11 (18B) : SEQ ID No. 11

ALETLLQNQQLLNSWGCRGRLVCYTSVRWNET

or

Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asn Ser Trp Gly Cys Arg Gly
1 5 10 15
Arg Leu Val Cys Tyr Thr Ser Val Arg Trp Asn Glu Thr
20 25 30

PEPTIDE No. 12 (19B) : SEQ ID No. 12

ALETLLQNQQLLNIWGCRGRLVCYTSVRWNET

or

Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asn Ile Trp Gly Cys Arg Gly
1 5 10 15
Arg Leu Val Cys Tyr Thr Ser Val Arg Trp Asn Glu Thr
20 25 30

PEPTIDE No. 13 (20B) : SEQ ID No. 13

ALETLLQNQQLLDLWGCRGRLVCYTSVRWNET

or

-Ala Leu Glu Thr Leu Leu Gln Asn Gln Gln Leu Leu Asp Leu Trp Gly Cys Arg Gly
1 5 10 15

Arg Leu Val Cys Tyr Thr Ser Val Arg Trp Asn Glu Thr
20 25 30

Sub. E3

PEPTIDE No. 14 (21B) : SEQ ID No. 14

LNQQRLLNSWGCKGRLVCYTSV

or

Leu Asn Gln Gln Arg Leu Leu Asn Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr
1 5 10 15

Thr Ser Val
20

PEPTIDE No. 15 (22B) : SEQ ID No. 15

RALETLLNQQRLLNSWGCKGRLVCYTSV

or

Arg Ala Leu Glu Thr Leu Leu Asn Gln Gln Arg Leu Leu Asn Ser Trp Gly Cys Lys
1 5 10 15

Gly Arg Leu Val Cys Tyr Thr Ser Val
20 25

PEPTIDE No. 16 (23B) : SEQ ID No. 16

RLNSWGCKGRLVCYTSV

or

Arg Leu Asn Ser Trp Gly Cys Lys Gly Arg Leu Val Cys Tyr Thr Ser Val
1 5 10 15

The synthetic peptides of formula (I), which are the subject of the present invention, can be obtained by solid phase synthesis according to conv ntional methods: R.B. Merrifield, *J. Amer. Chem. Soc. (1963)*, 85,

pp. 2149-2154 ; R.C. Sheppard, *in " Peptides 1971 ", Nesvadba H. (ed.) North Holland, Amsterdam, pp. 111* ; E. Atherton and R.L. Sheppard, *in " Solid phase peptide synthesis, a practical approach ", IRL PRESS, (1989), Oxford University Press, pp. 25-34.* As automatic synthesizer, it is possible to use the synthesizer " 9050 Plus Pep Synthesizer " from Millipore or an equivalent synthesizer.

The solid support used for the syntheses should be compatible with the technique and the chemistry used. For example, for a synthesis on the synthesizer " 9050 Plus pep. Synthesizer ", it is recommended to use a resin suitable for the so-called "continuous flow" technique; the PEG PS resins meet these criteria. These supports consist of an arm ("spacer") based on polyethylene glycol (PEG) situated between the functional group of the polystyrene beads and the point of attachment of the first amino acid. The nature of this point of anchorage may vary according to the C-terminal functional group chosen. In the present case, various PEG-PS resins were used.

The starting resin and the amino acids used as raw materials are products which are commercially available (PerSeptive-Biosystem, or Neosystem).

For the peptide synthesis, the following side chain protecting groups were used:

Amino acids	**Protecting group**
Arginine	Pentamethyl-2,2,4,6,7-dihydrobenzofuran-5-sulphonyl (Pbf)
Asparagine, Glutamine	Trityl (Trt)
Cysteine	Trityl (Trt) or Acetamidomethyl (Acm)
Serine, Threonine, Tyrosine	tert-Butyl ether (tBu)
Lysine, Tryptophan	tert-Butyloxycarbonyl (Boc)

The temporary protection of the primary amine functional group at the α position of the amino acids which is used is the 9-fluorenylmethyloxylcarbonyl (Fmoc) group. The deprotection is carried out with a 20% solution of piperidine in dimethylformamide.

For the coupling, an excess of diisopropylcarbodiimide (DIPCDI) and 1-hydroxybenzotriazole (HOBt) is preferably used.

After synthesis, the resin is washed with organic solvents (dimethylformamide, followed by dichloromethane), dried under vacuum and then treated with a trifluoroacetic acid (TFA)-based solution cooled to 0°C and containing appropriate " scavengers ". There may be used, for example, the K reagent containing 82% of trifluoroacetic acid, 5% of phenol, 5% of water, 5% of thioanisole and 3% of ethanedithiol.

After filtration of the resin, the synthetic peptides are precipitated and rinsed with ether.

The synthetic peptides are then purified by reversed phase liquid chromatography and their purity is determined by mass spectrometry. As solid phase, it is possible to use, for example, the Bondapak C-18 phase. The peptides are eluted by forming a linear gradient between two buffer solutions, the first which is essentially aqueous (for example water-TFA 0.1%) and the second which is rather organic (for example a mixture containing 60% acetonitrile, 39.92% water and 0.08% TFA). The pure fractions collected are combined, concentrated under vacuum and freeze-dried.

For the cyclization, the purified synthetic peptides are dissolved in an ammonium acetate solution (10 mM). The pH is adjusted to 8.5 by addition of 1 M ammonium hydroxide. The solution is vigorously stirred.

The cyclization is complete after 18 hours. The pH is then reduced to 6 by addition of acetic acid. The cyclized peptides are freeze-dried and then purified by reversed phase liquid chromatography as described above.

The immunoreactivity of the peptides of the invention was evaluated with the aid of sera from patients predominantly of Cameroonian origin infected with group O HIV-1 retroviruses. The various tests carried out demonstrated that the peptides of the invention, alone or in combination (compositions of peptides), make it possible to detect 100% of the sera infected with group O HIV-1 retroviruses.

The synthetic peptides of the invention therefore find application in immunological tests for the screening of infections due to group O HIV-1 retroviruses. It is also possible to use combinations of several synthetic peptides of formula I. These combinations, which may contain two or more peptides of formula I, also form part of the invention. Combinations containing peptides No. 1 (2B) and No.3 (4B) are preferred.

It is also possible to use synthetic peptides of formula (I) of the present invention in combination with group O HIV-1 recombinant peptides (recombinant proteins) as can be obtained by conventional methods and having the sequences described, for example in application EP 0,591,914. Such compositions are also within the scope of the present invention.

The synthetic peptides of the invention can also be used in combination with other HIV-2 and/or HIV-1 recombinant (recombinant proteins) or synthetic peptides, such as the peptides described in patent applications or patents EP 0,387,914, EP 0,239,425, EP 0,220,273 or EP 0,267,802. This list of patent applications or patents is not exhaustive and is given by way of example.

The compositions containing one or more synthetic peptides of

formula (I) and one or more HIV-1 or HIV-2 recombinant or synthetic peptides find application in diagnosis for the screening of patients infected with various HIV retroviruses. These compositions also form part of the present invention.

Immunoassay methods using one or more synthetic peptides of formula (I), alone or in combination with group O HIV-1 recombinant peptides or HIV-1 and/or HIV-2 recombinant or synthetic peptides, also form part of the invention.

The invention also relates to kits, for carrying out immunoassays, which include a peptide of formula (I) or a composition which contains at least one peptide of formula (I).

The following examples illustrate the invention and are given with no limitation being implied.

EXAMPLE 1 :

Preparation of a compound according to the invention; PEPTIDE No. 2 (3B)

LLSLWGCRGRLVCYTSVQWNET

or

Leu Leu Ser Leu Trp Gly Cys Arg Gly Arg Leu Val Cys Tyr Thr Ser Val Gln Trp Asn
1 5 10 15 20

Glu Thr
22

This peptide was synthesized on a solid phase. The technique developed in 1963 by Merrifield *(J. Am. Chem. Soc. (1963) 85, pp. 2149-2154)* consists in attaching the first amino acid onto a polymeric solid support (resin) by its acid functional group and in extending the peptide sequence from this first amino acid, the peptide being synthesized remaining anchored on the resin.

For the synthesis of peptide No. 2, there were used, as synthesizer, the synthesizer " 9050 Plus Pep Synthesizer " and as resin, the resin Fmoc Thr (OtBu) PEG PS.

The various steps of the synthesis are summarized in Table I below:

Table I

AMINO ACID RESIDUE	NH_2 PROTECTION	SIDE PROTECTION	COUPLING METHOD	EQ NUMBER - DURATION OF COUPLING
Glu	Fmoc	OtBu	DIPCDI/HOBt	5 eq - 30 min
Asn	Fmoc	Trt	DIPCDI/HOBt	5 eq - 30 min
Trp	Fmoc	Boc	DIPCDI/HOBt	5 eq - 30 min
Gln	Fmoc	Trt	DIPCDI/HOBt	5 eq - 30 min
Val	Fmoc		DIPCDI/HOBt	5 eq - 30 min
Ser	Fmoc	tBu	DIPCDI/HOBt	5 eq - 30 min
Thr	Fmoc	tBu	DIPCDI/HOBt	5 eq - 30 min
Tyr	Fmoc	tBu	DIPCDI/HOBt	5 eq - 30 min
Cys	Fmoc	Trt	DIPCDI/HOBt	5 eq - 30 min
Val	Fmoc		DIPCDI/HOBt	5 eq - 30 min
Leu	Fmoc		DIPCDI/HOBt	5 eq - 30 min
Arg	Fmoc	Pbf	DIPCDI/HOBt	5 eq - 30 min
Gly	Fmoc		DIPCDI/HOBt	5 eq - 30 min
Arg	Fmoc	Pbf	DIPCDI/HOBt	5 eq - 30 min
Cys	Fmoc	Trt	DIPCDI/HOBt	5 eq - 30 min
Gly	Fmoc		DIPCDI/HOBt	5 eq - 30 min
Trp	Fmoc	Boc	DIPCDI/HOBt	5 eq - 30 min
Leu	Fmoc		DIPCDI/HOBt	5 eq - 30 min
Ser	Fmoc	tBu	DIPCDI/HOBt	5 eq - 30 min
Leu	Fmoc		DIPCDI/HOBt	5 eq - 30 min
Leu	Fmoc		DIPCDI/HOBt	5 eq - 30 min

At the end of the synthesis, the resin was washed with dimethylformamide and then dichloromethane and dried under vacuum.

Next, the resin was treated with the K reagent (82% trifluoroacetic acid; 5% phenol; 5% water; 5% thioanisole; 3% ethanedithiol). Peptide No. 2 (3B), isolated by precipitation with the aid of diethyl ether, was then rinsed with the same solvent. 140 mg of peptide No. 2 (3B) were thus obtained.

Peptide No. 2 (3B) was then purified by reversed phase liquid chromatography. The Bondapak C-18 phase was used as solid phase. The peptide was eluted by forming a linear gradient between two buffer solutions, the first which is essentially aqueous (for example water-TFA 0.1%) and the second which is rather organic (for example a mixture containing: 60% acetonitrile, 39.92% water and 0.08% TFA). The pure fractions collected were combined, concentrated under vacuum and freeze-dried.

For the cyclization, the purified synthetic peptide thus obtained was dissolved in an ammonium acetate solution (10 mM). The pH was adjusted to 8.5 by addition of 1 M ammonium hydroxide. The solution was vigorously stirred. The cyclization was complete after 18 hours. The pH was then reduced to 6 by addition of acetic acid. The cyclized peptide was freeze-dried and then purified by reversed phase liquid chromatography as described above.

Preparation of a compound according to the invention: PEPTIDE No. 15 (22B)

This peptide was synthesized as peptide No. 2 (3B), but using as resin the resin FmocPAL PEG-PS.

The various steps of the synthesis are summarized in Table II

below:

Table II

AMINO ACID RESIDUE	NH_2 PROTECTION	SIDE PROTECTION	COUPLING METHOD	EQ NUMBER - DURATION OF COUPLING
Val	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Ser	Fmoc	tBu	DIPCDI/HOBt	5 eq - 45 mn
Thr	Fmoc	tBu	DIPCDI/HOBt	5 eq - 45 mn
Tyr	Fmoc	tBu	DIPCDI/HOBt	5 eq - 45 mn
Cys	Fmoc	Trt	DIPCDI/HOBt	5 eq - 45 mn
Val	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Leu	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Arg	Fmoc	Pbf	DIPCDI/HOBt	5 eq - 45 mn
Gly	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Lys	Fmoc	Boc	DIPCDI/HOBt	5 eq - 45 mn
Cys	Fmoc	Trt	DIPCDI/HOBt	5 eq - 45 mn
Gly	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Trp	Fmoc	Boc	DIPCDI/HOBt	5 eq - 45 mn
Ser	Fmoc	tBu	DIPCDI/HOBt	5 eq - 45 mn
Asn	Fmoc	Trt	DIPCDI/HOBt	5 eq - 45 mn
Leu	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Leu	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Arg	Fmoc	Pbf	DIPCDI/HOBt	5 eq - 45 mn
Gln	Fmoc	Trt	DIPCDI/HOBt	5 eq - 45 mn
Gln	Fmoc	Trt	DIPCDI/HOBt	5 eq - 45 mn
Asn	Fmoc	Trt	DIPCDI/HOBt	5 eq - 45 mn
Leu	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Leu	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Thr	Fmoc	tBu	DIPCDI/HOBt	5 eq - 45 mn
Glu	Fmoc	OtBu	DIPCDI/HOBt	5 eq - 45 mn
Leu	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Ala	Fmoc		DIPCDI/HOBt	5 eq - 45 mn
Arg	Fmoc	Pbf	DIPCDI/HOBt	5 eq - 45 mn

At the end of the synthesis, the resin was washed with dimethylformamide, followed by dichloromethane and dried under vacuum.

Next, the resin was treated with the K reagent (82% trifluoroacetic acid; 5% phenol; 5% water; 5% thioanisole; 3% ethanedithiol). The peptide No.7 (22B) isolated by precipitation with the aid of diethyl ether was then rinsed with the same solvent. 140 mg of peptide No. 15 (22B) were thus obtained.

Peptide No. 15 (22B) was then purified by reversed phase liquid chromatography and then cyclized, freeze-dried and purified as described above for peptide No. 2 (3B).

In the same manner, and using the appropriate resins and amino acids, the other compounds of the invention were synthesized.

Table III indicates the molecular weight of some peptides of formula (I), in non-cyclized form, evaluated by mass spectrometry.

Tabl III

Peptide No.	Molecular weight (Daltons)
1 (2B)	2512
2 (3B)	2583
3 (4B)	2528
4 (5B)	2586
5 (6B)	2527
9 (12B)	1772
10 (14B)	1799
11 (18B)	3752
12 (19B)	3778
13 (20B)	3780
14 (21B)	2538
15 (22B)	3222
16 (23B)	1941

EXAMPLE 2 :

Evaluation of the immunoreactivity of the peptides according to the invention by the immunoenzymatic test: Test No. 1

The sera used ESS, DUR, VAU and HAD are sera from French patients infected with group O HIV-1 retroviruses. The other serum samples from patients infected with group O HIV-1 retroviruses were obtained by the Yaoundé Pasteur Centre in Cameroon and were serotyped group O according to the serological algorithm described in *AIDS (1977), 11, pp 445-453.*

The HIV-negative sera (n=48) were obtained from healthy

volunteers.

The synthetic peptides used were dissolved in water at a concentration of 1 mg/ml (stock solution). For the solid phase sensitization step (coating), 110 µl of a solution at 2 µg/ml of each peptide (obtained by diluting the stock solution with 0.1 M carbonate buffer solution) were added to each well of the microtitre plates Microtiter™ (NUNC). After incubating overnight at room temperature, the microplates were first washed with a Tris-NaCl buffer solution pH 7.4 containing 0.1% of Tween® 20 and 0.001% sodium merthiolate, and then saturated with a PBS solution containing 0.5% of Régilait™ (dried skimmed milk). After aspirating the saturating solution, the plates were heated for 10 min at 50°C.

The serum samples were diluted 1/5 with a skimmed milk solution (citrate buffer supplemented with 0.01% of phenol red, 0.25% of chloroform and 0.25% of Kathon®), deposited in the wells of the plates and incubated for 30 min at 40°C.

After washing with a Tris-NaCl buffer solution pH 7.4 containing 0.1% of Tween® 20 and 0.001% of sodium merthiolate, 100 µl of a solution of conjugate consisting of horseradish peroxidase-labelled anti-human IgG and IgM goat antibodies, containing as preservative 0.01% of sodium merthiolate, in solution in a citrate buffer solution supplemented with 30% glycerol and 25% normal foetal calf serum, were added to each plate well and then the plates were incubated for 30 min at 40°C.

After washing with a Tris-NaCl buffer solution pH 7.4 containing 0.1% of Tween® 20 and 0.001% of sodium merthiolate, the colour was developed by adding, to each well, 100 µl of O-phenylenediamine in solution in hydrogen peroxide. The microplates were then incubated for 30 min at room temperature and in the dark. The coloured reaction was then stopped by addition of 100 µl of 4N sulphuric acid. The absorbance (A)

was determined at 490 and 620 nm.

The relative absorbance (A490-A620) read in each well is proportional to the immunoreactivity of each peptide. It indicates the ability of each peptide to react with the biological sample with which the test is carried out. The cut-off value was determined as being an absorbance equal to 0.15. It corresponds to the mean of the negative values (n=48) plus 12 standard deviations.

The reactivity of the peptides of the invention (peptide No. 3 (4B), peptide No. 2 (3B) and peptide No. 1 (2B), all in cyclized form), was compared with that of two synthetic peptides having, as sequence, a portion of the natural sequence of the envelope (env) of the VAU isolate (group O HIV-1 retrovirus) and comprising an immunodominant epitope of gp41.

These two peptides have the following sequence:

VAU 22 AA (SEQ ID NO: 21)

Leu Leu Asn Leu Trp Gly Cys Lys Asn Arg Ala Ile Cys Tyr Thr Ser Val Lys Trp Asn
1 5 10 15 20
Lys Thr
22

VAU 35 AA (SEQ ID NO: 22)

Arg Leu Leu Ala Leu Glu Thr Phe Ile Glu Glu Asn Glu Leu Leu Asn Leu Trp Gly Cys
1 5 10 15 20
Lys Asn Arg Ala Ile Cys Tyr Thr Ser Val Lys Trp Asn Lys Thr
25 30 35

For the study, these peptides were used in cyclized form. The results of this study are indicated in Table IV.

Table IV

SERUM	ABSORBANCE				
	PEPTIDE No. 3 (4B)	PEPTIDE No. 2 (3B)	PEPTIDE No. 1 (2B)	VAU 22 AA	VAU 35 AA
ESS*	>**	>	2.494	>	>
DUR*	>	>	>	0.118	0.872
HAD	>	0.518	0.041	0.789	0.871
VAU*	1.342	>	>	>	>
3935	>	0.893	0.307	0.138	0.227
6891	>	0.614	0.062	0.359	0.496
6512*	0.746	0.785	>	0.120	0.174
1105*	1.421	1.031	>	0.099	0.129
4021*	0.430	0.119	>	0.050	1.957
5969*	>	0.282	>	2.491	>
2700	>	0.274	>	>	>
5453	0.555	0.081	>	1.267	1.482
5931	>	>	>	0.202	2.225
3136	>	0.992	0.302	>	>
3653	1.352	>	0.044	1.441	1.322
2352	>	>	0.205	>	>
3016	>	>	0.243	>	>
3302	>	>	0.386	>	>
2294	>	>	0.447	>	>
3771	>	>	0.544	>	>
1581	>	>	>	1.112	0.894
5373	>	>	>	1.359	0.856
7443	>	>	>	0.920	0.574
3637	>	>	>	0.779	1.647
6295*	1.718	1.063	>	0.972	>
6689*	0.710	>	>	>	>
1754	>	>	>	1.263	1.948
4489*	>	>	>	1.318	1.718
4364	>	>	1.382	>	>
3884*	>	>	1.839	>	>
3529	>	>	1.803	>	>
3482	2.402	>	1.473	>	>
1702	>	>	1.162	>	>
6487	>	1.017	2.687	2.889	2.891
5164	>	>	>	>	>
5766*	>	>	>	>	>
3945	>	>	>	>	>

* serotypes / genotypes

** > = signal greater than the reading capacity of the spectrophotometer.

Table IV (continuation)

SERUM	ABSORBANCE				
	PEPTIDE No. 3 (4B)	PEPTIDE No. 2 (3B)	PEPTIDE No. 1 (2B)	VAU-22 AA	VAU 35 AA
4434	>	>	>	2.273	>
4288*	>	>	2.802	2.337	N.T.***
6782	>	2.091	2.462	2.190	2.214
2313	>	>	>	>	>
2312	>	>	>	>	>
1062	>	>	>	>	>
402	>	>	>	>	>
134	>	>	>	>	>
7120	>	>	>	>	>
7212	>	>	>	>	>
6976*	>	>	>	>	>
3600*	>	>	2.743	>	>
3236	>	>	>	>	>
3235	>	>	>	>	>
2551	>	>	>	>	>
5270*	>	>	>	>	>
5210	>	>	>	>	>
5149*	>	>	>	>	>
4477	>	>	>	2.511	>
3891	>	>	2.780	>	>
3627*	>	>	2.910	>	>
7258*	>	>	2.477	>	>
7007	2.136	2.334	>	>	2.151
6697	>	>	>	>	>
6998	>	>	>	>	>
6627	>	>	>	>	>
6198*	>	>	>	>	>
6165	>	>	2.714	>	>
7439	>	>	>	>	>
7297*	>	>	>	>	>
6111	>	>	>	>	>
625	>	>	>	>	2.885

* serotypes / genotypes

** > = signal greater than the reading capacity of the spectrophotometer.

*** Not tested

The results of Table IV demonstrate that peptide No. 3 (4B) exhibits the best performance with regard to that noted for the other peptides. This peptide allows the best discrimination between the sera of patients infected with group O HIV-1 retroviruses compared with the two peptides having a portion of the sequence of the VAU isolate corresponding to the immunodominant epitope of gp41. Moreover, peptide No. 2 (3B) and No. 1 (2B) of the invention are more immunoreactive than the VAU 22 AA peptide which comprises the same number of amino acids.

EXAMPLE 3 :

Evaluation, by an immunoenzymatic test, of the immunoreactivity of the peptides according to the invention: Test No. 2

The serum samples from patients infected with group O HIV-1 retroviruses were obtained by the Yaoundé Pasteur Centre in Cameroon and were serotyped group O according to the serological algorithm described in *AIDS (1977), 11, pp. 445-453*. A genotyped sample (Maryland) is obtained from the United States. These samples were previously diluted in negative human serum at the dilutions given in Table V, in order to have a sufficient volume for the different immunoreactivity tests.

The synthetic peptides used were dissolved in water at a concentration of 1 mg/ml (stock solution). For the solid phase sensitization step ("coating"), the procedure was carried out as described for Example 2.

The serum samples were diluted 1/5 with a skimmed milk solution (citrate buffer supplemented with 0.01% of phenol red, 0.25% of chloroform and 0.25% of Kathon®), deposited in the wells of plates and incubated for 30 min at 40°C.

After washing with a Tris-NaCl buffer solution pH 7.4 containing 0.1% of Tween® 20 and 0.001% of sodium merthiolate, 100 µl of a solution of conjugate consisting of horseradish peroxidase-labelled anti-human IgG and IgM goat antibodies, containing as preservative 0.01% of sodium merthiolate, in solution in a citrate buffer solution supplemented with 30% glycerol and 25% normal foetal calf serum, were added to each well of the plates and then they were incubated for 30 min at 40°C.

After washing with a Tris-NaCl buffer solution pH 7.4 containing 0.1% of Tween® 20 and 0.001% of sodium merthiolate, the colour was developed as described in Example 2.

The relative absorbance (OD) (A490-A620) read in each well is proportional to the immunoreactivity of each peptide. It indicates the ability of each peptide to react with the biological sample with which the test is carried out.

The reactivity of the peptides of the invention, peptides No. 10 (14B), No. 11 (18B), No. 12 (19B), No. 14 (21B), No. 15 (22B), No. 16 (23B) all in cyclized form, was compared with that of three homologous synthetic peptides having, as sequence, a portion of the natural sequence of the envelope (env) of a group O HIV-1 retrovirus. These peptides are two peptides derived from the VAU isolate - the peptide VAU 22 AA and the peptide VAU 35 AA - and the peptide MVP 5180 (designated "MVP 5180" in Table V). The peptides VAU 22 AA and VAU 35 AA (whose structure is indicated in Example 2) and the peptide MVP 5180 comprise an immunodominant epitope of gp41.

All these peptides were used in cyclized form. The sequence of the MVP 5180 peptide is the following:

MVP 5180 (SEQ ID NO:23)

Arg Leu Gln Ala Leu Glu Thr Leu Ile Gln Asn Gln Gln Arg Leu Asn Leu Trp Gly Cys
1 5 10 15 20

Lys Gly Lys Leu Ile Cys Tyr Thr Ser Val Lys Trp Asn Thr Ser
25 30 35

The results of this study are indicated in Table V.

Table V

SERUM	PEPTIDES *								
	No. 10	No. 11	No. 12	No. 14	No. 15	No. 16	MVP 5180	VAU 35 AA	VAU 22 AA
	ABSORBANCE (OD)								
4280 at 1/50	0.022	0.686	0.201	0.286	0.689	0.033	0.382	0.013	0.021
NGO at 1/50	0.067	0.335	0.193	0.157	0.315	0.110	0.184	0.055	0.040
NJEM at 1/100	0.032	0.811	0.391	0.277	0.939	0.025	0.146	0.159	0.024
MBASSI at 1/100	1.217	1.150	0.747	2.134	2.010	2.683	0.248	0.120	0.257
WANG at 1/50	0.698	0.234	0.124	2.397	2.680	1.290	0.075	0.025	0.041
258 OUDI at 1/100	0.587	0.373	0.226	0.764	1.184	1.692	0.116	0.058	0.100
DO15 at 1/100	1.613	0.859	1.286	3.357	3.693	3.038	0.673	0.036	0.075
DJOU at 1/100	1.268	0.482	0.419	1.998	2.088	2.166	0.203	0.022	0.042
3600 at 1/100	0.482	0.360	0.249	0.716	0.801	0.933	0.206	0.025	0.058
3613 at 1/100	1.108	0.837	0.773	1.508	1.627	1.679	0.478	0.250	0.396
6111 at 1/100	0.596	0.348	0.202	0.850	1.207	1.009	0.226	0.087	0.180
625 at 1/50	0.838	0.338	0.264	2.045	2.122	1.791	0.202	0.069	0.165
Maryland at 1/100	0.524	0.370	0.285	0.734	0.844	1.229	0.241	0.054	0.168
3653 at 1/10	0.347	0.337	0.247	0.072	0.380	0.406	0.401	0.021	0.310

SOLID PHASE* : PEPTIDE 2 µg/ml

For each peptide tested, the samples were arranged into four classes (a, b, c and d) corresponding to various levels of relative absorbance read at the wavelengths A492-A620 :

- - for a : OD < 0.100,
- - for b : 0.100 < OD < 0.500,
- - for c : 0.500 < OD < 1.000,
- - for d : OD > 1.000,

thus making it possible to evaluate the degree of immunoreactivity of the peptides. The most immunoreactive peptides are those for which the highest number of samples is found in the classes corresponding to the highest absorbance values.

The results are indicated in Table VI.

Table VI

CLASS	PEPTIDES *								
	No. 10	No. 11	No. 12	No. 14	No. 15	No. 16	MVP 5180	VAU 35 AA	VAU 22 AA
	Number of samples								
A	3	0	0	1	0	2	1	11	7
B	2	9	11	3	2	2	12	3	7
C	5	4	2	4	4	1	1	0	0
D	4	1	1	6	8	9	0	0	0

SOLID PHASE* : PEPTIDE 2 µg/ml

The results show that all the peptides of the invention tested achieve a better performance in immunoreactivity than the reference peptides of the prior art which are derived from natural isolates (MVP 5180, VAU). The peptides of the invention No. 15 (22B), No. 14 (21B), and No. 16 (23B) are found to be the most immunoreactive.

EXAMPLE 4 :

Evaluation of the immunoreactivity of the compositions containing peptides according to the invention by an immunoenzymatic test.

For this test, the procedure was carried out according to the protocol described in Example 2 and the same sera were used. The microplates used were sensitized either with peptide No. 1 (2B) cyclized, or with peptide No. 3 (4B) cyclized, or with a composition containing these two peptides (1:1 w/w). Into each well, there were deposited either 100 µl of a solution containing 2 µg/ml of peptide No. 1 (2B), or 100 µl of a solution containing 2 µg/ml of peptide No. 3 (4B), or 100 µl of a solution containing 1 µg/ml of peptide No. 1 (2B) and 1 µg/ml of peptide No. 3 (4B).

The results of this test are given in Table VII.

Table VII

SERUM	ABSORBANCE		
	PEPTIDE No. 1 (2B) (2 µg/ml)	PEPTIDE No. 3 (4B) (2 µg/ml)	PEPTIDE No. 1 (2B) (1µg/ml) + PEPTIDE No. 3 (4B) (1 µg /ml)
3529	1.803	>*	>
1105	>	1.421	>
3891	2.780	>	>
3235	>	>	>
2700	>	>	>
5931	>	>	>
3935	0.307	>	>
7443	>	>	>
1062	>	>	>
1754	>	>	>
3136	0.302	>	>
6891	0.062	>	>
5149	>	>	>
5270	>	>	>
2551	>	>	>
3600	2.743	>	>
6976	>	>	>
4489	>	>	>
6165	2.714	>	>
6198	>	>	>
6627	>	>	>
6998	>	>	>
6697	>	>	>
7258	2.477	>	>
3627	2.910	>	>
4477	>	>	>
3771	0.544	>	>
1702	1.016	>	>
2294	0.447	>	>
2352	0.205	>	>
3016	0.243	>	>
3302	0.386	>	>
3482	1.473	>	>
3653	0.044	1.322	1.105
4364	1.382	>	>
3637	>	>	>
4288	2.802	>	>
5969	>	>	>
258	>	>	>

* > = signal greater than the reading capacity of the spectrophotometer.

Table VII (continued)

SERUM	Absorbance		
	PEPTIDE No. 1 (2B) (2 µg/ml)	PEPTIDE No. 3 (4B) (2 µg/ml)	PEPTIDE No. 1 (2B) (1 µg/ml) + PEPTIDE No.° 3 (4B) (1 µg /ml)
6111	>	>	>
625	>	>	>
6853	>	2.769	>
3136	0.302	>	>
6689	>	0.710	>
6295	>	1.718	>
4021	>	0.430	2.381
3884	1.839	>	>
6512	>	0.746	>
6487	2.687	>	>
ESS	2.494	>	>
HAD	0.041	>	>
DUR	>	>	>

* > = signal greater than the reading capacity of the spectrophotometer.

The results of Table VII demonstrate that the compositions of the peptides of the invention, when used in diagnosis, allow the detection of all the sera from patients infected with group O HIV-1 retroviruses.